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Genomic Analysis of Epstein-Barr Virus in Nasal and Peripheral T-Cell Lymphoma: A Comparison With Nasopharyngeal Carcinoma in an Endemic Area

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The Epstein-Barr virus (EBV) is prevalent in nasal and peripheral T-cell lymphoma (NPTL) in Taiwan, where nasopharyngeal carcinoma (NPC) is endemic. In order to understand the pathogenesis of these two malignancies in this endemic area, genomic analysis of EBV in NPTL with comparison to NPC is important. We investigated the EBV subtype (types A and B), BamH-I "f" variant, and the Xho-I site mutant of the latent membrane protein-1 (LMP-1) gene in 19 cases of EBV-associated NPTL and in 30 cases of NPC. EBV DNA from three patients with infectious mononucleosis (IM) was simultaneously studied as representative of normal healthy carriers. Similar to NPC and IM, the EBV in NPTL was found to belong to the type A strain in the majority (18 of 19) of cases by analyzing the 3′ divergence of EBNA-2 genes. The extra restriction enzyme site in the BamHI-F region ("f" variant) of EBV DNA was frequently (15 of 30) demonstrated in NPC, but only rarely (1 of 19) was it detected in NPTL and IM (0 of 3). The Xho-I site mutant of the LMP-1 gene previously characterized in Chinese NPC also prevailed in NPTL and IM with an identical nucleotide sequence. No correlation exists between the EBV subtype and its variants. In conclusion, type A EBV is prevalent in Taiwanese NPTL, a finding much distinct from the dominance of type B virus in nonendemic European patients. The EBV genomes in NPTL are closely similar to those in IM or normal healthy carriers, but are distinct from NPC for the infrequency of the "f" variant. The prevalence of the LMP-1 mutant in this endemic region suggests that this EBV strain may confer a growth advantage role in the pathogenesis of these EBV-associated diseases. The rarity of the "f" variant in NPTL and its high frequency in NPC may explain the differential tumorigenesis of different EBV strains.

KEY WORDS: T-cell lymphoma, Epstein-Barr virus, genotype

INTRODUCTION

Epstein-Barr virus (EBV) is a human herpes virus that has ubiquitous distribution and causes widespread infection. It is implicated in the pathogenesis of infectious mononucleosis (IM), posttransplant lymphoma, African Burkitt's lymphoma (BL), and nasopharyngeal carcinoma (NPC) [Epstein et al., 1964; Hitt et al., 1989]. Recently, EBV has also been detected in the neoplastic cells of Hodgkin's disease [HD; Jarrett et al. 1991; Pallesen et al., 1991] and in nasal or peripheral T-cell lymphoma [NPTL; Jones et al., 1988; Harabuchi et al., 1990; Su et al., 1990, 1991]. Much effort has gone into investigating the genomic variation of EBV DNA among these diverse groups of EBV-associated human diseases in the hope of shedding light on the mechanism of EBV tumorigenesis.

EBV can establish latent infection and is maintained in a life-long carrier state. Three types of EBV latency have been established [Rowe et al., 1992]. In type I latency such as BL, only the EBV-encoded nuclear antigen-1 (EBNA-1) is expressed. In EBV-immortalized lymphoblastoid cells, a full spectrum of EBV latent genes including EBNA-1,2,3,4,5,6, LMP-1,2a,2b, and LP is expressed (type III latency). A type II latency with limited expression of EBNA-1 and LMPs was demonstrated in NPC, HD, and T-cell lymphoma [Hitt et al., 1989; Pallesen et al., 1991; Chen et al., 1993]. The expression of latent genes such as EBNA-2 and LMP-1 is associated

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with enhancing transformation ability [Wang et al., 1985; Rickinson et al., 1987], or it may impose a cytotoxic immune response to the EBV antigens expressed on the infected cells [Lee et al., 1993]. Both the transformation ability and the immune response may be related to the final emergence of EBV-associated human malignancies.

Analyzing the genomic status or the EBV strain based on the variation of these latent genes will provide valuable information on the mechanism of EBV tumorigenesis. Two subtypes of EBV genomes, i.e., type A and B strains based on the ability of the virus to transform human cells and on divergence in the 3′ sequences of EBNA-2,3,4 and, 6 genes, have been defined [Zimber et al., 1986; Maria et al., 1988; Sixbey et al., 1989; Kunimoto et al., 1992; Lung and Chang, 1992; Sample et al., 1994]. Studies of the distribution of EBV subtypes in human diseases indicated that the type A strain is predominant in tumor tissues obtained from Europe, Japan, and North America [Zimber et al., 1986; Kunimoto et al., 1992]. Type A EBV is also prevalent in healthy seropositive individuals in China and Hong Kong [Lung et al., 1992; Chen et al., 1992b]. In patients with immunodeficiency, either acquired or iatrogenic, the prevalence of the type B strain virus increases and may account for up to 50% of the cases [Young et al., 1987; Sculley et al., 1990; Boyle et al., 1991; Borisch et al., 1992; Sixbey et al., 1991; Kyaw et al., 1992]. A higher incidence of type B EBV has also been reported in nasal T-cell lymphoma from European and Japanese patients [Miyashita et al., 1991; Borisch et al., 1993]. Although NPTL is prevalent in the Chinese population [Chan et al., 1987; Ho et al., 1990a,b; Su, 1996; Su et al., 1991], the status of the EBV subtype in Chinese NPTL cases has not yet been documented.

Another candidate EBV gene, the latent membrane protein LMP-1 which plays an essential role in the transformation of B lymphocytes in vitro [Kaye et al., 1993], has been reported with a recurrent mutation at the Xho-I site of the LMP-1 gene resulting in the loss of a restriction enzyme site in Taiwanese NPC [Chen et al., 1992a]. Cloning and sequence analysis of this NPC strain have revealed that the nucleotide sequence at the Xho-I site of exon 1 of the LMP-1 gene in NPC changes from G to T, distinct from that of the prototype strain B95.8 [Menezes et al., 1975; Chen et al., 1992a]. This LMP-1 mutant may confer a growth advantage or escape from immune attack, leading to the final tumorigenesis [Chang et al., 1995].

Recently, detailed genetic studies have demonstrated that the prevalence of an extra enzyme site in the BamHI-F region or an "f" variant of EBV occurred almost exclusively in NPC patients and in high-risk healthy individuals with high titers of IgA against EBV-viral capsid antigen (VCA) in southern Chinese individuals [Lung et al., 1991]. Moreover, when NPC patients were in remission after radiotherapy, the "f" variant would be replaced by the prototype BamHI-F virus. The EBV "f" variant may have enhancing transformation function and could be another genomic marker specific for NPC [Lung et al., 1992; Liu et al., 1993]. The status of this "f" variant in Taiwanese NPTL, however, has not yet been clarified.

Taiwan is an endemic area of EBV-associated NPC and NPTL. In our experience, the two malignancies appear to be mutually exclusive in any individual patient. The reason for the differential tumorigenesis of EBV in different individuals remains totally unexplored. It is therefore important to analyze and compare the genomic status of the two EBV subtypes, the LMP-1 variant, and the BamHI-F genotype in these two EBV-associated human malignancies in this endemic area.

MATERIALS AND METHODS

Tissue Samples and Cell Lines

Samples from previously determined EBV-containing nasal and extranasal peripheral T-cell lymphomas (6 and 13 cases, respectively) as well as NPC (30 cases) were embedded and stored in a −70°C freezer until use. The histopathologic and immunophenotypic characteristics of the T-cell lymphoma cases have been described previously [Su, 1996; Su et al., 1991]. Specimens from IM patients (three cases) were used to represent the EBV strain in normal healthy Taiwanese. Two EBV-containing cell lines B95.8 and Jijoye, which represent EBV type A and type B strains, respectively, were used as controls.

Sample DNA Preparation

Tissue DNA was extracted from specimens of T-cell lymphoma, NPC, and IM. From each specimen, five to six 10 μm frozen sections were cut and treated with proteinase K (1 mg/ml) freshly prepared in 10 μM Tris HCl (pH 8.0), 10 μM EDTA, and 1% sodium dodecyl sulfate at 37°C overnight. The DNA was then purified by repeated extraction by phenol-chloroform-isoamyl alcohol (49:49:2) followed by ethanol precipitation.

Analysis of the EBV Subtype

The recognition of the EBV subtype was carried out by determining the 3′ sequence divergence of EBNA-2 gene by the polymerase chain reaction (PCR) method as described by Borisch et al., [1993]. The primers [gen1, gen2; Borisch et al., 1993] were designed from the EBNA-2 region and used as the outer primers (Table I). Briefly, 1 μg of genomic DNA was incubated with a 2 μl mixture of primers (gen1, gen2, both 10 pmol/μl), 1 U AmpliTaq™ DNA polymerase (from GeneAmp™ PCR kit, Perkin Elmer Cetus, Norwalk, CT), 10 μl synthesis buffer, 2 μl 2.5 mM dNTP, and 83 μl distilled water and mixed gently. The reaction mixture was heated to 94°C for 5 min to denature the DNA/DNA hybrid in a DNA thermal cycler (Perkin Elmer Cetus). Each amplification cycle included 1 min of denaturation at 95°C, 1 min of annealing at 55°C, and 1 min of extension at 72°C. The DNA amplification was run for 30 cycles. After the first PCR reaction, 10 μl of PCR products was mixed with EBNA2-A or EBNA2-B-specific primers (both 10 pmol/μl) for nested PCR to specifically separate the subtypes A and B. The nested PCR products were then electrophoresed in a 1.5% agarose gel, stained with ethidium bro-

TABLE I. List of Probes and Primers Used in This Study

	Nucleotide sequence positions
(A) EBNA2	
gen 1 5′AGGGATGCCTGGACACAA 3′	48810–48827
gen 2 5′GTGCTGGTGCTGCTGGTGG 3′	49410–49391
EBNA2 subtype 1	
A1 5′TCTTGATAGGGATCCGCTAGGATA 3′	48839–48862
A2 5′ACCGTGGTTCTGGACTATCTGGATC 3′	49335–39311
Probe 5′CTCTGTCACAACCGAGGCTTACC 3′	49048–49070
EBNA2 subtype 2	
B1 5′CATGGTAGCCTTAGGACATA 3′	Borisch et al, 1993
B2 5′AGACTTAGTTGATGCCCTAG 3′	
Probe 5′AGGCCTACTCTTCCTCAACCCAG 3′	
(B) BamH-1F	
F1 5′GGAACTGAGCCAGTAGGATA 3′	149–173
F2 5′AATGTTCTGCAGGGTAACGG 3′	980–960
(C) LMP-XhO-1	
X1 5′AGAAACACGCGTTACTCT 3′	169081–169098
X2 5′TACAATGCCTGTCCGTGCA 3′	169577–169559

mide, visualized under UV light, and then Southern blot transferred to a Zeta-probe nylon membrane (AMF, Canno, Meriden, CT). DNA from type A EBV strain B95.8 and type B EBV strain Jijoye was included as controls in each run. The membrane was then prehybridized and hybridized with specific probes (Table I).

BamH-I "f" Variant Analysis

The "f" variant analysis was based on the presence of an extra enzyme site at the BamHI-F fragment region of EBV DNA. In this study, PCR using the designed primers (Table I) was employed to detect the EBV BamHI "f" variant. The primer pair were BamHI-F1 and BamHI-F2 designed from the BamHI-F region. The conditions of PCR reaction were the same as those described above. After PCR reaction, 10 μl of PCR products was mixed with 2 μl of BamHI (10 U/μl) restriction enzyme (BRL, Grand Island, New York), 2 μl of 10× reaction buffer-2 (BRL), and 6 μl of distilled water, and then incubated in 37°C for 2 hr. The products were analyzed from the extra BamHI-F site by electrophoresis in 1.5% agarose gel, stained with ethidium bromide, visualized under UV light, and then Southern blot transferred to a Zeta-probe nylon membrane (AMF). After baking at 80°C for 2 hr, the membrane was prehybridized and then hybridized with specific probes (kindly provided by Dr. Mei-Ying Liu, Graduate Institute of Microbiology, College of Medicine, National Taiwan University).

Xho-I Site Analysis of the LMP-1 Gene

The LMP-1 mutant or NPC-EBV strain was studied by Xho-1 restriction enzyme analysis of the PCR-amplified products and subsequent cloning and sequencing of this product obtained from T-cell lymphoma, NPC, and IM patients.

Xho-I restriction enzyme site analysis of LMP-1. By using the designed PCR primers (Table I), the LMP-1 gene would have a Xho-I site in prototype B95.8 EBV. However, this restriction enzyme site was lost in the LMP-1 mutant or NPC strains as reported previously [Chen et al., 1992a]. Genomic DNA (1 μg) was added to 100 μl of the PCR mixture. The reaction mixture contained a final concentration of 1× PCR synthesis buffer, 0.05 mM dNTP, 2 U of Taq polymerase (Perkin Elmer Cetus), and 1 pmol/μl of each primer. The nucleotide sequence of the primer pair (X_1, X_2) is shown in Table I. Each amplification cycle included 1 min of denaturation at 95°C, 1 min of annealing at 55°C, and 1 min of extension at 72°C. The DNA amplification was run for 32 cycles. Followed by a primer extension step at 72°C for 5 min, 43 μl of PCR product was mixed with 2 μl of Xho-1 restriction enzyme (10 U/μl; BRL), 5 μl of 10× reaction buffer 2, and was incubated in 37°C for 5 hr. After the reaction, 20 μl of digested product was analyzed in 1.5% agarose gel, stained with ethidium bromide, visualized under UV light, Southern blot transferred to a Zeta-probe nylon membrane (AMF), and then prehybridized and hybridized with a specific probe (the 497 bp fragment of Xho-I digested PCR product of B95.8 cell line was used as the probe).

Cloning and sequencing of the LMP-1 gene. The PCR-amplified product of the LMP-1 gene was sampled further for cloning and sequencing to examine the detailed sequence variation. For preparing the cloning vector, the pUC18 plasmid DNA (100 ng) was digested with 2 U Sma-1 enzyme (BM, Mannheim, Germany) at 37°C for 1 hr and purified by phenol:chloroform (1:1) extraction. The plasmid DNA was precipitated with 3–4 volumes of 100% ethanol at −70°C for 15 min. After washing with 70% ethanol, the pelleted DNA was redissolved in distilled water. The PCR amplification product generated from the X_1 and X_2 primer pair (Table I) was ligated into the pUC18 vector. The ligation mixture contained 100 ng of digested plasmid DNA with Sma-1, the amplified PCR product (17 ng), 2 U of T4 DNA ligase (BM), and 10× buffer (BM) at a final volume of 10 μl. The mixture was incubated in 25°C for at least 6 hr until transformation. Then 200 μl of competent cells were mixed with 10 μl of ligation mixture and swirled gently on ice for 30 min, and then heat shocked in 42°C for 45 sec. The transformation mixture was transferred to 1

TABLE II. Comparison of EBV Subtype, "f" Variant, and Xho-I Site Mutant of LMP-1 in T-Cell Lymphoma (NPTL), NPC, IM (or Representative of Normal Healthy Taiwanese), and Prototype B95.8*

	No. case tested	EBV subtype	BamHI "f" variant	Xho-I site LMP-1 mutant
(1) Prototype B95.8		A	F	P
(2) NPTL	19	A (18)	F (18)	M (17)
		A + B (1)	f (1)	P (2)
(3) NPC	30	A (25)	F (15)	M (28)
		A + B (5)	f (15)	P (2)
(4) IM	3	A (3)	F (3)	M (3)

*F, prototype F fragment without BamHI restriction enzyme site; f, variant F fragment with BamHI rstriction enzyme site; P, prototype of LMP-1 gene containing the Xho-I site; M, mutant of the LMP-1 gene. The numbers in parentheses indicate number of cases tested positive.

ml LB broth at 37°C for 1 hr. After the reaction the mixture was then spread onto the LB Amp+ (ampicillin) agar plate and incubated at 37°C for 8 hr. After incubation, the white colonies were selected and innoculated in Amp+(ampicillin) LB broth. The plasmid was purified by a plasmid purification kit (QIAGEN, Helden, Germany) according to the manufacturer's instructions. To check the insert, the plasmid DNA was digested with EcoRI and Hind III enzyme (BRL) on 1% agarose gel.

For sequence analysis of the PCR products, purified plasmid DNA 3–5 μg was denatured by adding 0.1 volumes of 2 M NaOH and 2 mM EDTA and incubated for 30 min at 37°C. The mixture was neutralized by 0.1 volumes of 3 M sodium acetate (pH 4.5–5.5). The DNA was then precipitated with 2–4 volumes of ethanol at −70°C for 15 min, washed with 70% ethanol, then the pelleted DNA was redissolved in 6 μl of distilled water. Two microliters of sequenase reaction buffer and 2 μl of primer (Sequenase TM Version 2.0 DNA sequencing kit USB Cleveland, OH) were added for annealing in 37°C for 15 min. The reaction was then chilled on ice and centrifuged briefly. During cooling, four tubes containing 2.5 μl of each termination mixture, ddATP, ddCTP, ddGTP, ddTTP, were prepared and kept at room temperature. The annealed DNA mixture was added with 1 μl of 0.1 M DTT, 2 μl of 1× labeling mixture (USB), 0.5 μl of ^{35}S dAPT, and 0.5 μl sequencing enzyme, and incubated at room temperature for 2–5 min. Then 3.5 μl of labeling reaction mixture was transferred to each tube containing ddATP, ddCTP, ddGTP, ddTTP, mixed gently, and incubated at 37°C for 5 min. The reaction was stopped by adding 4 μl of stop solution. The reaction samples were heated at 75°C for 2 min immediately before running on a urea-denatured polyacrylamide gel.

RESULTS

The results of analysis on the prevalence of the EBV subtype, "f" variant, and LMP-1 mutant in NPTL, NPC, and IM are summarized in Table II.

EBV Subtype Analysis

The analysis of EBV subtype was studied by determining the 3′ divergence of EBNA-2 using the designed PCR primers (Table I). The presence of a 497 bp product represents the type A strain and a 150 bp product represents the type B strain. The EBV DNA from B95.8 and Jijoye was used as controls of type A and type B EBV, respectively. The results revealed that type A EBV strain was detected in all 18 cases of T-cell lymphoma; the remaining patient had a coinfection of type A and type B EBV (Fig. 1A). In NPC, type A EBV strains was detected in 25 of 30 cases and a coinfection of type A and type B EBV strain was detected in the remaining 5 cases (Fig. 1B). Type A EBV strain was also detected in all three cases of IM. Therefore, the type A EBV strain was predominant in Taiwanese NPTL, NPC, and IM in this series. The result is different from the European and Japanese reports of a predominance of type B EBV strain in NPTL patients [Miyashita et al., 1991; Borisch et al., 1993].

BamHI "f" Variant Analysis

The "f" variant analysis was based on the presence of an extra BamHI restriction enzyme site at the F fragment region of EBV DNA. By using the designed PCR primers (Table I), the PCR amplification product of the prototype BamHI-F fragment is 831 bp in size. After cleavage with the BamHI restriction enzyme, two fragments of 702 bp and 129 bp in size were yielded, respectively, for the "f" variant (Fig. 2). The PCR products amplified from specimens containing prototype EBV could not be cleaved by BamHI, and could be readily distinguished from those harboring the "f" variant. Among the 19 cases of NPTL we studied, only 1 case (5.3%) belonged to the "f" variant (Fig. 2a), strikingly distinct from the 50% (15 of 30 cases) in NPC (Fig. 2b) ($P < 0.05$). All three cases of IM had prototype BamHI-F EBV (Fig. 2C).

Xho-I Restriction Enzyme Site Analysis of the LMP-1 Gene

The LMP-1 mutation analysis was based on the Xho-I restriction analysis of the LMP-1 exon 1 gene using the designed PCR primers (Table I). A 497 bp product flanking the Xho-I site region of LMP-1 was detected by PCR analysis. The prototype B95.8 EBV DNA can be cleaved by the Xho-I restriction enzyme into two fragments of 340 bp and 157 bp, respectively. However, the Xho-I site is absent in the NPC-EBV strain. The data revealed that the Xho-I site was absent in 17 of 19 (90%) cases of NPTL (Fig. 3A), in 28 of 30 (93%) cases of NPC (Fig. 3B), and in all three cases of IM (Fig. 3C). The nucleotide sequence of this PCR product was further

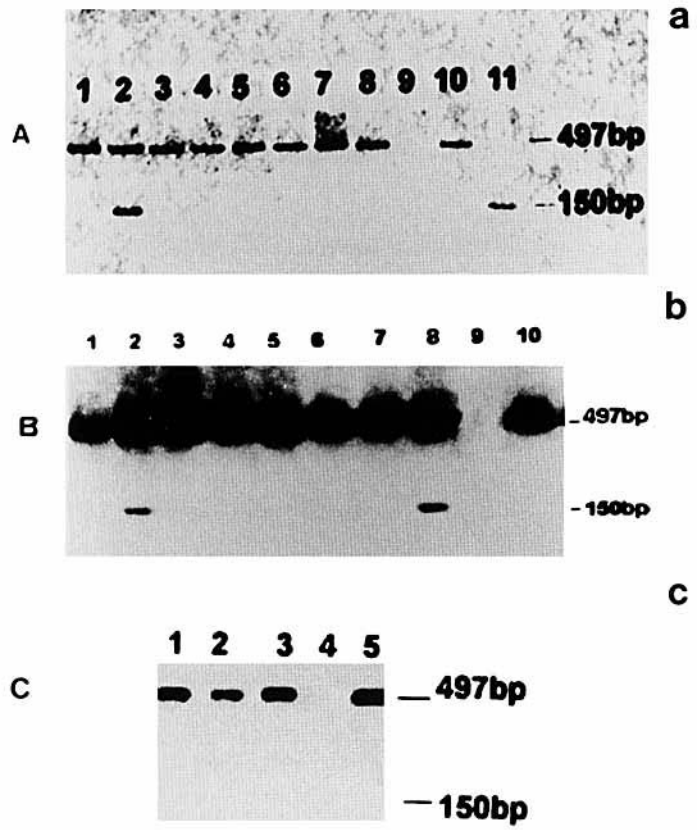

Fig. 1. Determination of EBV subtypes by Southern blot hybridization of the PCR products. **A: lane 1:** EBV strain of NPTL; **lanes 3–8:** type A; **lane 2:** type A/B (497 bp represents type A EBV strain and 150 bp represents type B EBV strain), **lane 9:** H_2O as a negative control; **lane 10:** B95.8 cell line as a control for type A EBV strain; **lane 11:** Jijoye line as a control for the type B EBV strain. **B: lanes 1, 3–7:** NPC cases, type A; **lanes 2, 8:** type A/B; **lane 9:** H_2O; **lane 10:** B95.8. **C: Lanes 1–3:** type A EBV strain was also detected in IM cases; **lane 4:** H_2O; **lane 5:** B95.8.

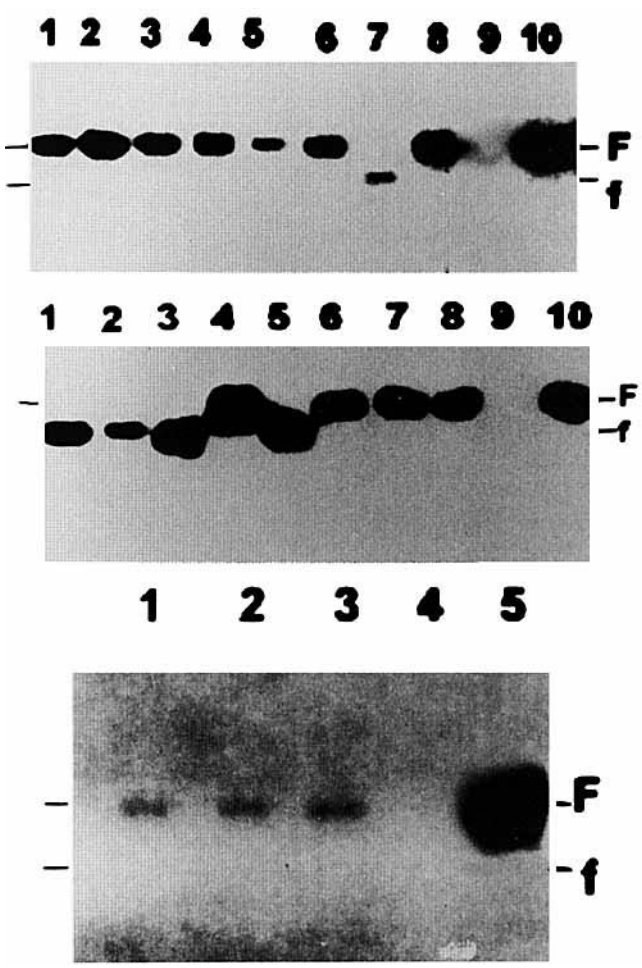

Fig. 2. Southern blot hybridization of the PCR products of the BamHI-F region of the EBV gene from T-cell lymphoma, NPC, and IM. **a:** T-cell lymphoma cases, "f" variant in **lane 7** and the others **(lanes 1–6, 8** belonged to prototype F fragments; **lane 9:** H_2O; **lane 10:** B95.8. **b:** NPC cases, "f" variant was observed in **lanes 1,2,3,5** and prototype F fragment in **lanes 4,6,7,8; lane 9:** H_2O; **lane 10:** B95.8 DNA. **c: Lanes 1-3:** IM cases and prototype F fragment; **lane 4:** H_2O; **lane 5:** B95.8.

studied in six isolates of NPTL, one NPC, two IM, and one B95.8. The data revealed that the Xho-I site sequence of LMP-1 in all six cases of NPTL (Fig. 4a) was identical to that in NPC (Fig. 4b) and IM (Fig. 4d) but different from the prototype B95.8 (Fig. 4c). In all cases of NPTL, NPC, and IM the nucleotide sequence at position 169426 changed from G (in the B95.8 strain) to T. The other nucleotide sequence remained 95% homologous to the LMP-1 gene of the B95.8 strain. This variation of the LMP-1 gene was also reflected in the change of the predicted amino acid sequence of the LMP-1 protein: amino acid 85 changed from ILe to Leu, amino acid 13 from Arg to Leu, 46 from Asp to Asn, 84 from Lys to Gly, 25 from Leu to ILe, as well as 3 from His to Arg.

DISCUSSION

Recent studies revealed that two distinct entities of EBV-associated human malignancies, i.e., NPC and NPTL, are prevalent in southern China, Hong Kong, and Taiwan [Chan et al., 1987; Ho et al., 1990a,b; Su et al., 1991; Su, 1996; Chen et al., 1992]. This observation raises several important questions. Firstly, what are the responsible factors (genetic, environmental, or virologic) which determine the prevalence of two EBV-associated human malignancies in an endemic area? Secondly, are there different strains of EBV in different diseases? The different EBV strains may affect the transformation ability or the cytotoxic immune response, leading to different human diseases. The possible genomic variation of EBV among the diverse groups of EBV-associated human diseases in this region should be investigated.

In the current analysis, we studied the EBV subtype in T-cell lymphoma, with comparison to NPC and IM patients. The type A EBV strain was demonstrated in nearly all cases of Taiwanese NPTL, similar to that in NPC and healthy seropositive individuals. Our observation differs from the data reported from Europe and Japan, where a type B EBV strain was predominant in NPTL [Miyashita et al., 1991; Borisch et al., 1993]. Since

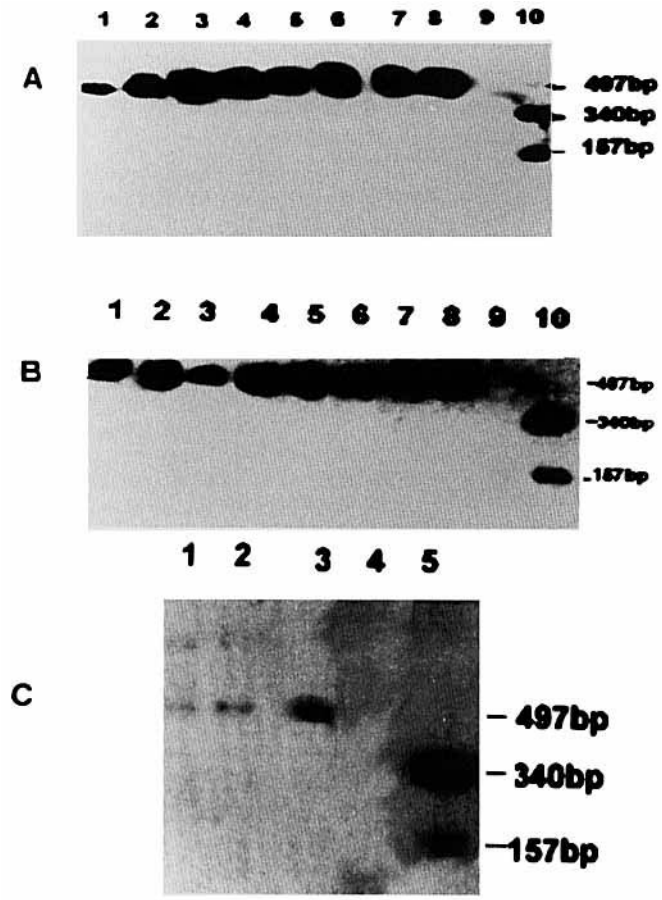

Fig. 3. Analysis of the Xho-I enzyme site of the EBV LMP-1 gene in T-cell lymphoma, NPC, and IM samples. Southern blot hybridization of the Xho-I enzyme-digested PCR products. **A:** T-cell lymphoma. **Lanes 1–8:** Loss of the Xho-I site and a 497 bp DNA band was detected. The prototype LMP-1 gene in B95.8 **(lane 10)** revealed two bands of 340 and 157 bp; **lane 9:** H_2O as a negative control. **B:** The Xho-I site of the LMP-1 gene was also lost in NPC cases **(lanes 1–8). Lane 9:** H_2O; **lane 10:** B95.8. **C: Lanes 1–3:** IM cases and the Xho-I site was lost; **lane 4:** H_2O; **lane 5:** B95.8.

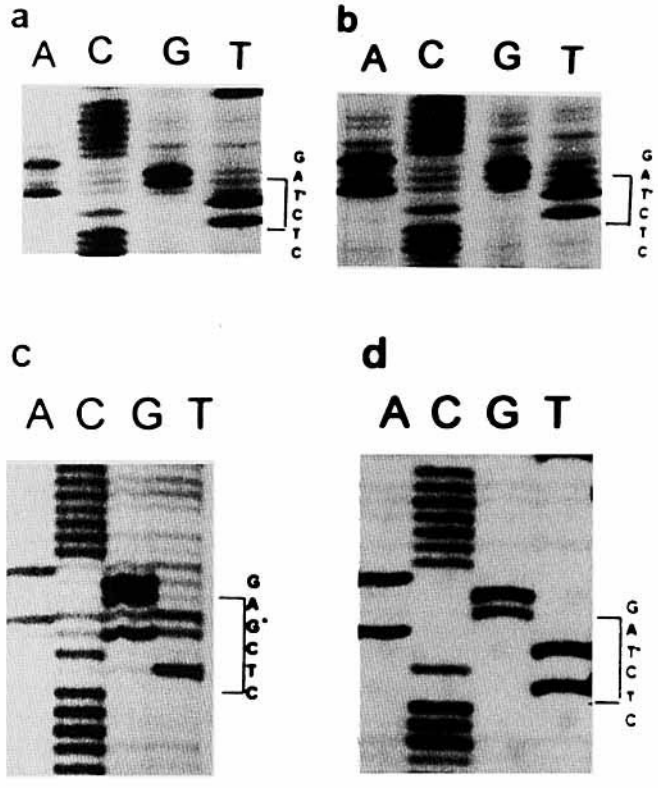

Fig. 4. Nucleotide sequence analysis of the 169081-169577 region of the EBV LMP-1 gene. **a:** T-cell lymphoma. **b:** NPC. **c:** B95.8 cell line. **d:** IM cases. A "T" instead of "G" at position 169426 could be detected in T-cell lymphoma, NPC, and IM samples, distinct from that in the B95.8 cell line.

EBV-containing NPTL is prevalent or endemic in the Chinese population and occurs only rarely in Western countries [Borisch et al., 1993; Su et al., 1991; Su, 1996], the discrepancy in the prevalence of EBV subtype in T-cell lymphoma in different regions may have important implications. According to past observations, type B EBV usually infects the tumors occurring in patients with immunodeficiency. The prevalence of a low-transforming type B virus in European NPTL patients may therefore suggest that these patients have an underlying immunodeficient state, similar to the prevalence of the type B EBV strain in patients with acquired immunodeficiency and HD [Boyle et al., 1991]. In immunocompetent patients and in endemic areas, a type A virus with high transformation capacity is usually prevalent [Kunimoto et al., 1992; Lung et al., 1992]. Our observation of a predominant presence of type A EBV in NPTL in Taiwan strengthens further the previous assumption of the implication of EBV subtype in tumorigenesis.

The extra presence of an enzyme site in the EBV BamHI-F region ("f" variant) has been documented in most cases of NPC in southern Chinese individuals [Lung et al., 1991], and also in healthy individuals with elevated IgA antibody levels against EBV capsid antigen, suggesting that the "f" variant may be specifically associated with NPC. In this study, we confirmed the previous observation and revealed that the "f" variant was detected in a high percentage (15 of 30) of patients with NPC. However, the "f" variant was only infrequently (1/19) detected in NPTL patients. Since both NPC and NPTL harbored type A strain EBV, the difference in the genotype of the BamHI-F region might be implicated in the differential tumorigenesis of EBV. The BamHI-F region contains several open reading fragments [Hudson et al., 1985; Takada et al., 1986] which may affect the translation of BamHI-H [Pearson et al., 1987]. It is therefore possible that the BamHI-F region plays a selective role in the final emergence of different types of EBV-associated malignancies in different individuals.

The EBV LMP-1 protein is essential for growth transformation of B lymphocytes in vitro, and is the only EBV-encoded protein which can transform rodent fibroblasts in culture [Wang et al., 1985; Baichwal and Sugden, 1988; Kaye et al., 1993]. Previous reports have identified a unique variant of EBV, or the so-called NPC

strain marked by the absence of an Xho-I site, in the N-terminal region of LMP-1 [Chen et al., 1992; Abdel-Hamid et al., 1992]. This study, however, revealed that up to 90% of EBV infection in T-cell lymphoma also showed absence of the Xho-I site in exon 1 of the LMP-1 gene, closely similar to the 93% in NPC patients. Furthermore, the sequence analysis revealed the same nucleotide change from G to T at position 169426 of the LMP-1 gene, which is identical to that in NPC and IM. Taken together, our results suggest that the LMP-1 mutant, or the so-called NPC strain, may be predominantly present in the Chinese population. The mutation in the LMP-1 gene of the NPC strain may exhibit a more potent transforming ability or may confer a growth advantage as compared to the wild-type LMP-1 in B95.8. Alternatively, the mutations in the LMP-1 gene may result in changes in the antigenicity of the LMP-1 protein and escape the T-cell-mediated elimination, leading to the final emergence of EBV-associated human malignancies.

This study revealed several interesting and important observations. The type A EBV strain is predominant in T-cell lymphoma in Taiwan, much different from the prevalence of the type B virus in NPTL in Japan and Europe where EBV-associated NPTL is not endemic. The predominant presence of the LMP-1 mutant in the Chinese population may be related to the prevalence of two important EBV-associated human malignancies in this region. Finally, the different distribution of the "f" variant in NPC and T-cell lymphoma may explain the differential tumorigenesis or selective tissue tropism of EBV.

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